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The limit of the genetic adaptation to herbicides in freshwater phytoplankton and the adaptation photosynthetic cost

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Background: One of the most important anthropogenic impacts on freshwater aquatic ecosystems is the continuous increase of herbicide concentrations, which impacts on the structure of phytoplankton communities.

Objectives: The maximum adaptation of two green microalgae (*Dictyosphaerium chlorelloides* and *Chlamydomonas reinhardtii*) and a cyanobacterium (*Microcystis aeruginosa*) to two widely used herbicides: glyphosate (N-(phosphonomethyl)glycine) and diuron (C₉H₁₀Cl₂N₂O, 3-(3,4-diclorofenil)-1,1-dimetilurea) was explored. Additionally, the cost of the herbicide resistance on photosynthesis and growth rate was evaluated.

Methods: We used an eco-evolutionary approach (ratchet protocol) to explore the maximum genetic adaptation. We characterised the photosynthetic performance by oxygen production and PSII chlorophyll a fluorescence.

Results: A dose of 1 µM diuron or 40 ppm glyphosate completely inhibited the growth of *M. aeruginosa* and *D. chlorelloides*, whereas *C. reinhardtii* growth was completely abolished at 2 µM diuron or 90 ppm glyphosate. However, an increase in resistance to both herbicides was achieved in certain populations during the ratchet experiment. *Microcystis aeruginosa* and *D. chlorelloides* were able to adapt up to 8 µM diuron and 80 ppm glyphosate, whereas *C. reinhardtii* adapted up to twice these herbicide concentrations. The photosynthetic performance was generally lower in the resistant than in the wild-type strains in the three species. These results suggest that increasing concentrations of these herbicides in freshwater bodies could induce the selection of herbicide resistant mutants in phytoplankton communities but showing lower primary production than original populations.